CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE:

2.1. Insecticide resistance in *Culex quinquefasciatus*:

Chemical insecticides are used to control mosquito vectors since many decades in order to suppress the transmission of various vector-borne diseases. Broadly, four different classes of insecticides have been approved for application against mosquito vectors in and around the globe. The use of synthetic insecticides in the past have proved fruitful in combating the deadly mosquito-borne diseases like dengue, malaria, filariasis, Chikungunya *etc*., when medical treatment for those diseases were scarce. Even in the present scenario, there are many mosquito-borne diseases lacking vaccination and proper medical treatment thereby putting the entire focus alone on the control of mosquito population through use of chemical insecticides.

With excessive and unrestrained use of the insecticides against vector population, the first report on resistance to DDT in *Aedes* mosquitoes was in the early 1950s (Giullin and Peters, 1952). Since then, there are several reports on resistance to chemical insecticides in the mosquito vectors from different corners of the world. The resistance status reported by different research studies is an important issue and a major topic of concern because as the resistant population grows, the mosquito vectors might become inevitable and without proper medical facilities the vector-borne diseases may become difficult to handle causing many fatalities and economic loss to mankind.

Resistance against synthetic insecticides in *Culex quinquefasciatus* are reported from various regions of the world. Unlike the resistance reported in *Aedes* mosquitoes, many researchers support the idea of resistance development in *Cx. quinquefasciatus* due

to the indirect exposure of the vector to insecticides that were applied and targeted against *Aedes* mosquito vectors. Because of lack of proper knowledge and training of the manpower, insecticides directed against *Aedes* mosquitoes are still being applied on the drains irrespective of their natural habitats being tree holes, discarded tyres, plastic containers and all other such habitats that contain clear water for the *Aedes* mosquitoes to breed in. *Cx. quinquefasciatus* is a highly opportunistic mosquito with an ability to breed in any form of temporary body of stagnant water that is a bit dirty, opaque and rich in organic content apart from natural breeding habitats like muddy drains, cemented channels, sewers, earthern pools of water, small puddles and plastic containers with dull opaque water.

Cx. quinquefasciatus is an indoor resting, anthropophilic mosquito that feed on human and avian blood thereby forming a zoonotic bridge between human and aves. Resistance to insecticides in *Cx. quinquefasciatus* is therefore a prime problem which if been unable to tackle, would bring about major health issues as WHO has claimed *Cx. quinquefasciatus* to be one of the most important and highly adaptive urban vectors which may cause several vector-borne diseases in days to come (WHO, 2017b). Rapid and unplanned urbanization in the developing tropical and sub-tropical countries combined with the growing threat of resistance development in the vector population hints upon some major problem to be faced in the near future pertaining to mosquito vectors and different vector-borne diseases those mosquitoes carry.

The present review work compiles information on insecticide resistance in *Cx. quinquefasciatus* from different parts of the world since the year 2000. Though there were various reports of insecticide resistance in *Cx. quinquefasciatus* earlier to the above-

mentioned year, yet to constrain the length and information inclusion of the review, data from the year 2000 onwards are included here. This might be helpful in providing a current trend of insecticide resistance and in understanding the evolution of resistance development in *Cx. quinquefasciatus* in the past twenty years of insecticide use.

2.1.1. Resistance to organophosphates:

The organophosphate class of insecticides is used widely not only in the vector management programs but also in the agricultural sector to combat agricultural pests in India and around the globe. Such reliance on the use of organophosphate insecticides has led to the development of resistance in the pest population against different insecticides belonging to this class. Likewise, there are several reports on resistance to organophosphate insecticides in *Cx. quinquefasciatus* larvae and adults (Table 3).

Table 3: Insecticide resistance status against Organophosphates in *Cx. quinquefasciatus.*

Sl. no.	Insecticides used	Life stages	Mortality (%)	Status	Country	References
1	Malathion	Adults	22	R	Sri Lanka	Karunaratne and Hemingway, 2001
2	Fenitrothion	Adults	93.5-96	IR	Thailand	Somboon *et al.*, 2003
	Malathion	Adults	47.1-100	R/IR/S		
3	Fenitrothion	Adults	———	———	Malaysia	Nazni *et al.*, 2005
	Malathion	Adults	0	R		
4	Malathion	Adults	0	R	India (Andhra Pradesh)	Mukhopadhyay *et al.*, 2006
5	Fenitrothion	Adults	21.2-100	R/IR/S	Thailand	Sathantriphop *et al.*, 2006
	Malathion	Adults	100	S		
6	Chlorpyrifos-methyl	Adults	71-100	R/IR/S	Benin	Corbel *et al.*, 2007

	Malathion	Adults	95-100	IR/S		
7	Malathion	Adults	84.5-100	IR/S	Thailand	Thanispong *et al.*, 2008
8	Malathion	Adults	9.75	R	India (Chhattisgarh)	Raghavendra *et al.*, 2011
9	Malathion	Adults	33.3	R	India (Gujarat)	Raghavendra *et al.*, 2011
10	Malathion	Adults	27.50	R	India (Uttar Pradesh)	Kumar *et al.*, 2011
11	Malathion	Adults	100	S	Zambia	Norris and Norris, 2011
12	Naled	Adults	3.2-12	R	Louisiana, United States	Gordon and Ottea, 2012
13	Malathion	Adults	73.33	R	Malaysia	Chen *et al.*, 2013
	Fenitrothion	Adults	100	S		
14	Malathion	Adults	4.44-100	R/IR/S	Malaysia	Low *et al.*, 2013a
15	Malathion	Adults	44-100	R/IR/S	Ghana	Kudom *et al.*, 2013
16	Malathion	Adults	30	R	Pakistan	Tahir *et al.*, 2013
17	Malathion	Adults	0-53	R	United States	Richards *et al.*, 2017
18	Pirimiphos-methyl	Larvae	0.04*	____	India (Delhi)	Ansari *et al.*, 2004
19	Malathion	Larvae	109.62-140.31*	R	Malaysia	Nazni *et al.*, 2005
	Temephos	Larvae	26.3*	R		
20	Temephos	Larvae	100	S	India (Andhra Pradesh)	Mukhopadhyay *et al.*, 2006
	Fenthion	Larvae	100	S		
	Fenitrothion	Larvae	8	R		
	Malathion	Larvae	14	R		
21	Malathion	Larvae	1.498*	R	India (Rajasthan)	Bansal and Singh, 2007
	Fenitrothion	Larvae	0.0719*	R		
	Fenthion	Larvae	0.0817*	R		
	Temephos	Larvae	0.0056*	R		

22	Temephos	Larvae	0.0162*	R	India (Jodhpur)	Suman *et al.*, 2010
		Larvae	0.0086*	R	India (Bikaner)	
		Larvae	0.0048*	R	India (Jamnagar)	
		Larvae	0.0136*	R	India (Bathinda)	
	Fenthion	Larvae	0.0792*	R	India (Jodhpur)	
		Larvae	0.0470*	R	India (Bikaner)	
		Larvae	0.0461*	R	India (Jamnagar)	
		Larvae	0.0238*	R	India (Bathinda)	
23	Temephos	Larvae	0.0031-0.0044*	R	La Reunion	Tantely *et al.*, 2010
	Malathion	Larvae	0.0012-0.0028*	R		
	Chlorpyrifos	Larvae	0.0018-0.0098*	R		
24	Temephos	Larvae	30	R	India (Uttar Pradesh)	Kumar *et al.*, 2011
25	Temephos	Larvae	0.0008-0.0083*	R	India (Bengaluru)	Shetty *et al.*, 2013
	Fenthion	Larvae	0.00012-0.8492*	R		
26	Temephos	Larvae	2.8-71	R	India (Delhi)	Thomas *et al.*, 2013
		Larvae	82.1	R	India (Uttar Pradesh)	
		Larvae	81.5	R	India (Haryana)	
27	Malathion	Larvae	0.1*	——	India (Pune)	Gokhale *et al.*, 2013
28	Malathion	Larvae	0.045-1.65*	R	Malaysia	Low *et al.*, 2013b
29	Temephos	Larvae	0.002 – 0.56*	R	Brazil	Amorim *et al.*, 2013
30	Chlorpyrifos	Larvae	0.56	R	Nigeria	Anogwih *et al.*, 2015
	Pirimiphos-methyl	Larvae	30.47	R		
31	Temephos	Larvae	24-77	R	Southern Louisiana	Delisi *et al.*, 2017

32	Chlorpyrifos	Larvae	0.0056*	____	Bukina Faso	Skovmand and Sanago, 2018
	Malathion	Larvae	0.043*	____		
	Temephos	Larvae	0.0011-0.0068*	____		

*Lethal concentration (LC_{50}) value in ppm; R: resistance; IR: intermediate resistance; S: susceptible

Temephos is the most widely used organophosphate larvicide worldwide and is approved by WHO for use against larvae of mosquito vectors – *Aedes sp., Anopheles sp.*, and *Culex sp*. In India, the National Vector Borne Disease Control Programme (NVBDCP) has also approved and provided a diagnostic dose for the use of temephos for mosquito larval control. *Cx. quinquefasciatus* - a vector of lymphatic filariasis in the South-East Asian countries, is reported to be resistant to temephos since many decades. Thereby imposing a threat on the extensive use of this insecticide and a need for developing and discovering alternative chemical substances that are comparatively more efficient in suppressing the larval growth and proliferation and in turn transmission of lymphatic filariasis and other vector-borne diseases.

In India, resistance to temephos in *Cx. quinquefasciatus* is reported from several states in the past twenty years. Bansal and Singh (2007) reported temephos resistant *Cx. quinquefasciatus* populations in Jodhpur (Rajasthan, India) with a LC_{50} value of 0.0056 ppm and immediately after two years resistance to temephos in *Cx. quinquefasciatus* was again reported from Jodhpur, Bikaner, Jamnagar and Bathinda with RR_{50} (resistance ratio) values of 10.8, 5.73, 3.2, 9.06 respectively in Rajasthan (Suman *et al*., 2010). Similar reports on temephos resistance in *Cx. quinquefasciatus* are recorded from Uttar Pradesh with 30% mortality rate (Kumar *et al*., 2011) and 82.1% mortality rate (Thomas *et al*., 2013), Bengaluru in Karnataka with an RR_{50} value of 10.37 (Shetty *et al*., 2012),

Delhi with 71% mortality and Haryana with mortality rate of 81.5 (Thomas *et al*., 2013). However, the sole susceptible population to temephos in India was reported from Andhra Pradesh with cent percent larval mortality (Mukhopadhayay *et al.,* 2006). Apart from India, temephos resistance in *Cx. quinquefasciatus* has also been reported from various countries like Malaysia (Nazni *et al.,* 2005), Japan (Kasai *et al*., 2007), La Reunion (Tantely *et al*., 2010), Brazil (Amorim *et al*., 2013), Southern Lousiana (Delisi *et al*., 2017) and Burkino Faso (Skovmand and Sango 2018).

Malathion, an organophosphate is known for its toxicity not only to mosquito vectors and other insect pests but also to higher vertebrates including humans. The insecticide malathion is used both as a larvicide and adulticide and is considered as one of the most potent chemicals against mosquito vectors. Unfortunately, due to unrestrained use of malathion, the mosquito vector *Cx. quinquefasciatus* has acquired resistance to this insecticide as evident from various data reported from different parts of India. There are reports on malathion resistance from Andhra Pradesh (Mukhopadhyay *et al*., 2006), Rajasthan (Bansal and Singh 2007), Chhattisgarh with 9.75% mortality rate in adults, Gujarat with 33.33% adult mortality (Raghavendra *et al*., 2011), Uttar Pradesh (Kumar *et al*., 2011) and Pune (Gokhale *et al*., 2013). Similar reports on resistance development from other countries *viz*., Sri Lanka, Iran, Benin, La Reunion, Ghana, Malaysia, Pakistan, United States and Burkino Faso are also recorded in many studies (Table 3). On the contrary, there are few cases of susceptible *Cx. quinquefasciatus* populations to malathion in Malaysia (Nazni *et al*., 2005), Thailand (Sathantriphop *et al.,* 2006) and Zambia (Norris and Norris, 2011).

Apart from temephos and malathion, the organophosphate class of insecticides contain various other insecticides that are applied against the mosquito vectors in different regions of the world *i.e.,* fenitrothion, fenthion, chlorpyrifos *etc*. Similar to the resistance report against temephos and malathion, resistance of *Cx. quinquefasciatus* to other organophosphorus insecticides have been reported both in India and worldwide (Table 3) (Figure 7). However, there are few cases on the susceptible status of *Cx. quinquefasciatus* to an organophosphate - fenitrothion from Andhra Pradesh, India where cent percent mortality in the larval population was observed after exposure to the insecticide (Mukhopadhyay *et al.,* 2006), Japan (Kasai *et al.,* 2007) and Thailand with 98 – 100 percent adult mortality rate (Sathantriphop *et al.,* 2006). The organophosphate group of insecticides, works by attacking the acetylcholinesterase enzyme and are therefore, known as acetylcholinesterase inhibitor. Resistance of *Cx. quinquefasciatus* to this group of insecticides might become a major issue of concern due to its worldwide application including India, being the only efficient insecticide group targeted against mosquito larvae. Moreover, there are high risks of cross resistance with other insecticides that have similar mode of action and target-site on the mosquito vectors. The phenomenon of cross resistance in the insecticides will render many groups of insecticides to become inefficient in controlling a vector population at a given time. This simultaneous failure of insecticide groups to counter upon mosquito vectors during major disease outbreak would result in uncontrolled spread of disease pathogen and thereafter, high fatality rate in poor and developing countries where medical facilities and infrastructure remain low and scarce.

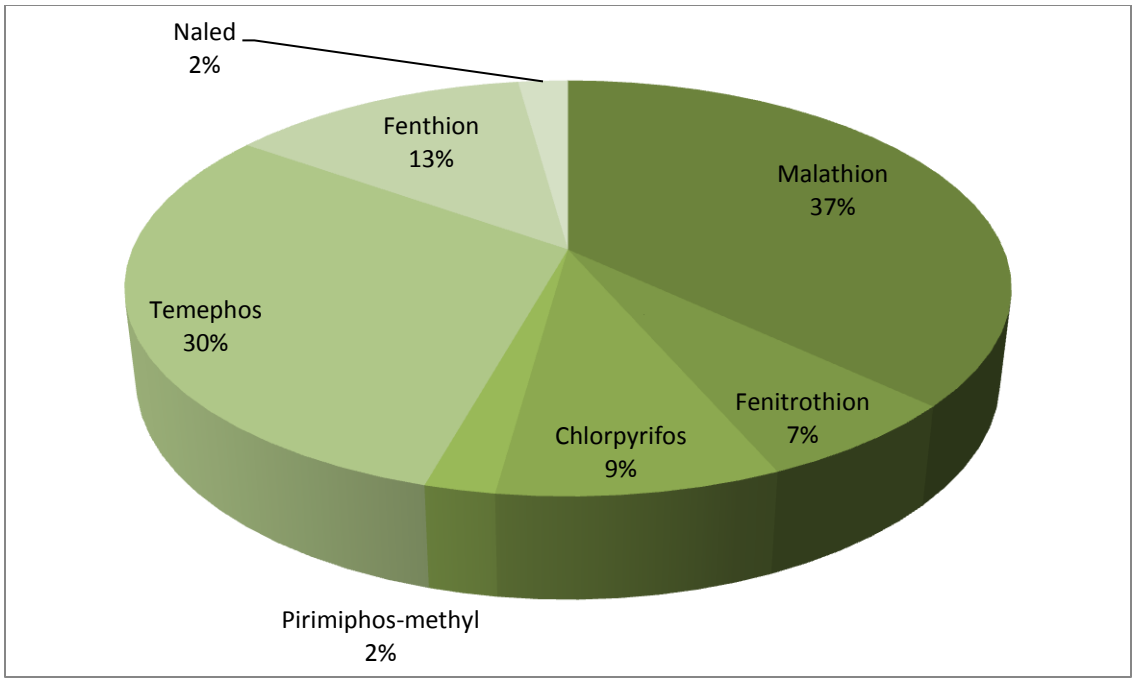


Figure 7: Resistance percentage against organophosphate insecticides in *Cx. quinquefasciatus*.

2.1.2. Resistance to synthetic pyrethroids:

Synthetic pyrethroids are applied against different mosquito vectors and also for various agricultural pests including lepidopteran pests. The rapid action of synthetic pyrethroids on the pests' nervous system makes it an efficient group of insecticides to be used against a wide array of insects and other arthropod pests. Synthetic pyrethroids are derived from pyrethrins that is found in pyrethrum extract of *Chrysanthemum sp*. (Dong, 2007). This insecticide group is further sub-divided into two categories based on their chemical structure and mode of action on the target site. The difference in their chemical structure is the presence of an α-cyano group at phenylbenzyl alcohol position in Type II pyrethroids and the same lacking in Type I pyrethroids. Type I pyrethroids act by causing repetitive discharge in response to a single stimulus whereas Type II pyrethroids act by

causing membrane depolarization subsequently followed by a decline in the nerve action potential (Dong, 2007).

Though synthetic pyrethroids are used against both adult and larval mosquitoes, it is more commonly applied as an adulticide due to its rapid action compared to other insecticides. Synthetic pyrethroids, moreover, are the primary insecticide group used till date in insecticide treated bed nets and LLINs though carbamates are also used in a lesser frequency. As such, resistance to this insecticide group is a prime issue as there is no potential substitute to synthetic pyrethroids in terms of insecticide treated bed nets (ITNs). Apart from ITNs, synthetic pyrethroids form the main composition of household mosquito repellent creams, oils, fumigants, sprays, coils, *etc*. Resistance to synthetic pyrethroids in *Cx. quinquefasciatus* is obvious owing to the wide and frequent use of this insecticide in the domestic areas, *Cx. quinquefasciatus* being anthropophilic and indoor resting. Therefore, there are many studies and reports of resistance to various synthetic pyrethroids in *Cx. quinquefasciatus* (Table 4).

Table 4: Insecticide resistance status of *Cx. quinquefasciatus* against synthetic pyrethroids and pyrroles.

Sl. no.	Insecticides used	Life stages	Mortality (%)	Status	Country	References
1	Deltamethrin	Adults	50.4	R	Malawi	Adams *et al.*, 2002
	Alphacypermethrin		56.4	R		
	Cyfluthrin		63.1	R		
2	Cyfluthrin	Adults	28.59-29.95	R	Malaysia	Nazni *et al.*, 2005
	Lambdacyhalothrin		34.38-36.43	R		
	Permethrin		78.15-79.82	R		

3	Permethrin	Adults	96.5-100	IR/S	Thailand	Somboon *et al.,* 2003
	Deltamethrin		97.6	IR		
	Lambdacyhalothrin		100	S		
	Etofenprox		66.7-70.4	R		
4	Deltamethrin	Adults	11	R	Thailand	Sathantriphop *et al.,* 2006
	Permethrin		10.1	R		
5	Permethrin	Adults	35-93	R/IR	Benin	Corbel *et al.,* 2007
6	Deltamethrin	Adults	11.1	R	Tanzania	Mosha *et al.,* 2008
	Chlorfenapyr		30.8	R		
7	Permethrin	Adults	67.4-80.6	R/IR	Thailand	Thanispong *et al.,* 2008
8	Permethrin	Adults	1	R	Sri Lanka	Wondji *et al.,* 2008
9	Deltamethrin	Adults	30-35	R	Pakistan	Tahir *et al.,* 2009
10	Deltamethrin	Adults	12-54.7	R	Thailand	Sathantriphop *et al.,* 2006
11	Deltamethrin	Adults	96.2-100	IR/S	India (Assam)	Sarkar *et al.,* 2009
12	Permethrin	Adults	80-96	R/IR	Zambia	Norris and Norris, 2011
	Deltamethrin		97-98	IR		
13	Deltamethrin	Adults	44.9	R	India (Chhattisgarh)	Raghavendra *et al.,* 2011
14	Deltamethrin	Adults	75	R	India (Gujarat)	Raghavendra *et al.,* 2011
15	Deltamethrin	Adults	96.66	IR	India (Uttar Pradesh)	Kumar *et al.,* 2011
	Cyfluthrin		98.33	IR		
	Permethrin		95.83	IR		
	Lambdacyhalothrin		98.33	S		
16	Resmethrin	Adults	5.8-96.7	R/IR	Louisiana, United States	Gordon and Ottea, 2012
17	Deltamethrin	Adults	19.4	R	Zanzibar, Tanzania	Jones *et al.,* 2012
	Lambdacyhalothrin		24	R		

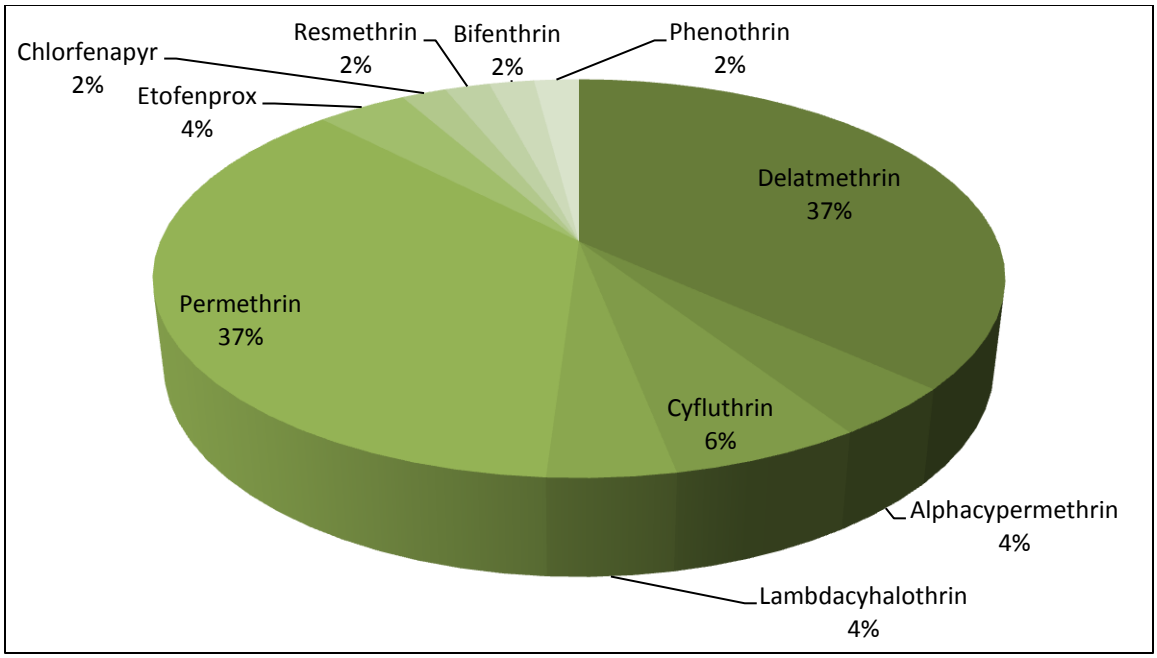
	Permethrin		14	R		
18	Permethrin	Adults	66.67	R	Malaysia	Chen *et al.,* 2013
	Deltamethrin		33.33	R		
	Cyfluthrin		26.67	R		
	Lambdacyhalothrin		80	IR		
	Etofenprox		93.33	IR		
19	Deltamethrin	Adults	91-97	IR/S	Ghana	Ekloh *et al.,* 2013
20	Permethrin	Adults	36.67-100	R/IR/S	Malaysia	Low *et al.,* 2013b
21	Alphacypermethrin	Adults	24.61	R	India (Maharastra)	Karlekar *et al.,* 2013
	Deltamethrin		72.73	R		
22	Permethrin	Adults	91.11-95.56	IR	Malaysia	Wan-Norafikah *et al.,* 2013
23	Permethrin	Adults	4-100	R/IR/S	Ghana	Kudom *et al.,* 2013
24	Permethrin	Adults	39-96	R/IR	Ghana	Kudom *et al.,* 2015
	Deltamethrin		23-58	R		
25	Permethrin	Adults	4-24	R	Benin	Yadouletan *et al.,* 2015
	Deltamethrin		24-48	R		
26	Deltamethrin	Adults	12.9-93.4	R/IR	Thailand	Yanola *et al.,* 2015
	Permethrin		11.4	R		
27	Permethrin	Adults	45.6 - 85.5	R/IR	Thailand	Boonyuan *et al.,* 2016
28	Bifenthrin	Adults	6-88	R/IR	United States	Richards *et al.,* 2017
	Deltamethrin		17-98	R/IR		
	Etofenprox		0-28	R		
	Permethrin		0-84	IR		
	Phenothrin		0-52	R		

29	Permethrin	Adults	43	R	Burkina Faso	Skovmand and Sanago, 2018
30	Deltamethrin	Adults	20	R	Cameroon	Nchoutponen *et al.*, 2019
	Permethrin		18	R		
31	Permethrin	Larvae	0.8-7.5*	R	Alabama	Xu *et al.*, 2005
32	Alphamethrin	Larvae	0.0002*	____	India (Rajasthan)	Bansal and Singh, 2007
	Deltamethrin		0.00007*	____		
	Fanvalerate		0.0112*	____		
33	Deltamethrin	Larvae	0.0008*	R	Mysore	Fakoorziba *et al.*, 2009
34	Permethrin	Larvae	0.5-8.2*	____	United States of America	Liu *et al.*, 2009
35	Permethrin	Larvae	0.01*	R	United States of America	Li and Liu, 2014
36	Permethrin	Larvae	0.0025-0.0029*	R	La Reunion	Tantely *et al.*, 2010
	Deltamethrin	Larvae	0.0019-0.0038*	R		
37	Deltamethrin	Larvae	0.00062*	____	India (Mysore)	Kumar *et al.*, 2011
	Lambdacyhalothrin	Larvae	0.00001*	____		
38	Chlorphenapyr	Adults	100	S	India (Chhattisgarh)	Raghavendra *et al.*, 2011
39	Chlorphenapyr	Adults	100	S	India (Gujarat)	Raghavendra *et al.*, 2011
40	Lambdacyhalothrin	Larvae	0.0006-0.0041*	R	India (Bengaluru)	Shetty *et al.*, 2012
	Deltamethrin	Larvae	0.0016-0.0065*	R		
41	Permethrin	Larvae	0.049-0.803*	R	Malaysia	Low *et al*., 2013b
42	Lambdacyhalothrin	Larvae	0.0002*	____	India (Pune)	Gokhale *et al.*, 2013
43	Permethrin	Larvae	1.62-1.78*	R	Malaysia	Wan-Norafikah *et al.*, 2013
44	Deltamethrin	Larvae	0.18*	S	Thailand	Yanola, 2015

45	Permethrin	Larvae	0.033-0.041*	____	Bukina Faso	Skovmand and Sanago, 2018
46	Meperfluthrin	Larvae	12.37*	R	China	Yuan *et al.*, 2019
	Dimefluthrin		15.8*	R		
	Heptafluthrin		40.11*	R		
	Metofluthrin		62.46*	R		
	Transfluthrin		66.43*	R		

*LC_{50} value in ppm; R: resistance; IR: intermediate resistance; S: susceptible

In India, resistance to synthetic pyrethroids in *Cx. quinquefasciatus* larvae is reported from different cities in various states (Table 4). The researchers have provided LC_{50} values and RR values of few synthetic pyrethroids like deltamethrin, lambdacyhalothrin, alphamethrin and fanvalerate thereby, reporting the resistance level of synthetic pyrethroids in *Cx. quinquefasciatus* from Rajasthan (Bansal and Singh, 2007), Mysore (Harish Kumar *et al.*, 2011), Bengaluru (Shetty *et al.,* 2013), Gorakhpur and Pune (Gokhale *et al.,* 2013). On the other hand, intermediate resistance to susceptible status against four commonly used synthetic pyrethroids were also reported in adult *Cx. quinquefasciatus* from Uttar Pradesh (Kumar *et al.,* 2011) and against deltamethrin from Assam (Sarkar *et al.,* 2009). Thereafter, various reports on resistance in *Cx. quinquefasciatus* was reported against deltamethrin in Chhattisgarh and Gujarat (Raghavendra *et al.,* 2011) and against alphacypermethrin, and deltamethrin in Nagpur (Karlekar *et al.,* 2013). Likewise, there are several reports on resistance to synthetic pyrethroids from different corners of the world (Table 4) (Figure 8).



.Figure 8: Resistance percentage against Synthetic Pyrethroids in *Cx. quinquefasciatus*.

Apart from direct exposure to synthetic pyrethroids in the domestic household applicants and resting on the pyrethroid-treated bed nets, indirect and a higher concentration of exposure of *Cx. quinquefasciatus* to synthetic pyrethroids is also due to the agricultural run-off. Synthetic pyrethroids are applied extensively in the agricultural sector and the washed away residues accumulate in the adjoining drains where the lymphatic filariasis vector *Cx. quinquefasciatus* dwell. Moreover, this scenario aggravates the already serious problem of synthetic pyrethroids resistance in *Culex* mosquitoes. As such, taking care of the household mosquitocidal products and insecticide treated bed nets (ITNs) alone will not be enough to solve the present problem of resistance. However, this condition if unchecked will pose a serious threat of vector-borne diseases in the near future as we already know about synthetic pyrethroids being

the only insecticide group to be used in ITNs. Furthermore, its rapid mode of action might be hard to be substituted by any other insecticide group in immediate future.

2.1.3. Resistance to Organochlorines:

The most commonly used organochlorine in the control of mosquito vectors include DDT and dieldrin. Ever since the first report on DDT resistance in mosquito in 1950s (Giullin and Peters, 1952), there has been several cases of resistance to chemical insecticides which might be because of the overexploitation of this insecticide class in vector control programmes and the agricultural sector. Few years later in 1952, resistance to DDT was reported in *Cx. quinquefasciatus* from a village in Delhi, India as well (Gopalakrishnan and Veer, 2018). This resistance trend was immediately followed with new reports on resistance in the same mosquito vector from other three Indian cities – Pune, Nagpur, and Patna against DDT and dieldrin (Gopalakrishnan and Veer, 2018). Unfortunately, with a history of resistance dating almost 70 years back, organochlorine insecticides are still used against the insect vectors of medical importance though being banned for use in the agricultural sectors. The continuous use of DDT led to resistance development even in the last 20 years and there are several reports on resistance to DDT in India and other countries worldwide (Table 5).

Table 5: Insecticide resistance status of *Cx. quinquefasciatus* against Organochlorines.

Sl. no.	Insecticides used	Life stages	Mortality (%)	Status	Country	References
1	DDT	Adults	2-68.8	R	Thailand	Somboon *et al.*, 2003
2	DDT	Adults	100	S	Malaysia	Nazni *et al.*, 2005
3	DDT	Adults	0	R	India (Andhra Pradesh)	Mukhopadhyay *et al.*, 2006

4	DDT	Adults	0	R	Thailand	Sathantriphop *et al.*, 2006
5	DDT	Adults	5-54	R	Benin	Corbel *et al.*, 2007
	Dieldrin	Adults	86-99	IR/S		
6	DDT	Adults	2.2-8.3	R	Thailand	Thanispong *et al.*, 2008
7	DDT	Adults	1	R	Sri Lanka	Wondji *et al.*, 2008
8	DDT	Adults	11.9-50	R	India (Assam)	Sarkar *et al.*, 2009
9	DDT	Adults	28.33	R	India (Uttar Pradesh)	Kumar *et al.*, 2011
10	DDT	Adults	13-69	R	Zambia	Norris and Norris, 2011
11	DDT	Adults	0	R	India (Chhattisgarh)	Raghavendra *et al.*, 2011
12	DDT	Adults	3.3	R	India (Gujarat)	Raghavendra *et al.*, 2011
13	DDT	Adults	3.2	R	Tanzania	Jones *et al.*, 2012
14	DDT	Adults	33.33	R	Malaysia	Chen *et al.*, 2013
	Dieldrin		33.33	R		
15	DDT	Adults	12.3	R	India (Maharastra)	Karlekar *et al.*, 2013
16	DDT	Adults	1.3-95.7	R/IR	Ghana	Kudom *et al.*, 2013
17	DDT	Adults	2.22-40	R	Malaysia	Low *et al.*, 2013
18	DDT	Adults	13-75	R	Ghana	Kudom *et al.*, 2015
19	DDT	Adults	4-12	R	Benin	Yadouletan *et al.*, 2015
20	DDT	Larvae	0.51*	——	India (Pune)	Gokhale *et al.*, 2013
21	DDT	Larvae	0.725-4.205*	R	Malaysia	Low *et al.*, 2013
22	DDT	Larvae	2.67*	R	Bukina Faso	Skovmand and Sanago, 2018
23	Dieldrin	Larvae	0.0014-0.009*	R	La Reunion	Tantely *et al.*, 2010

*LC_{50} value in ppm; R: resistance; IR: intermediate resistance; S: susceptible

The adults of *Cx. quinquefasciatus* were reported to show severe resistant status against DDT with zero percent mortality in Andhra Pradesh (Mukhopadhyay *et al.,* 2006). Similar resistance status with cent percent mortality was reported from Raipur as well in the year 2011 and Kheda, Gujarat (3.3% mortality) (Raghavendra *et al.,* 2011). *Cx. quinquefasciatus* was also found to exhibit high resistance level against DDT from Assam (11.9-50% mortality) (Sarkar *et al.,* 2009), Uttar Pradesh with 28.33% mortality (Kumar *et al.,* 2011) and Nagpur (12.3% mortality) (Karlekar *et al.,* 2013). Likewise, larvae of *Cx. quinquefasciatus* were reported to be resistant to the insecticide DDT from Gorakhpur and Pune (Gokhale *et al.,* 2013).

The hurdle of organochlorine resistance is not only confined to India but is widespread in various countries (Figure 9) with reports on DDT resistance in larval population of *Cx. quinquefasciatus* in Burkina Faso (Skovmand and Sango, 2018) and resistance against dieldrin in La Reunion (Tantely *et al.,* 2010). Similarly, the adults of lymphatic filariasis vector *Cx. quinquefasciatus* have been reported to possess 100% mortality against DDT and dieldrin in both laboratory and semi field environment in Malyasia (Nazni *et al.,* 2005). However, in the year 2013 there were reports on DDT resistance in *Cx. quinquefasciatus* from Malaysia with a low mortality percent of 33.33 (Low *et al.,* 2013b).

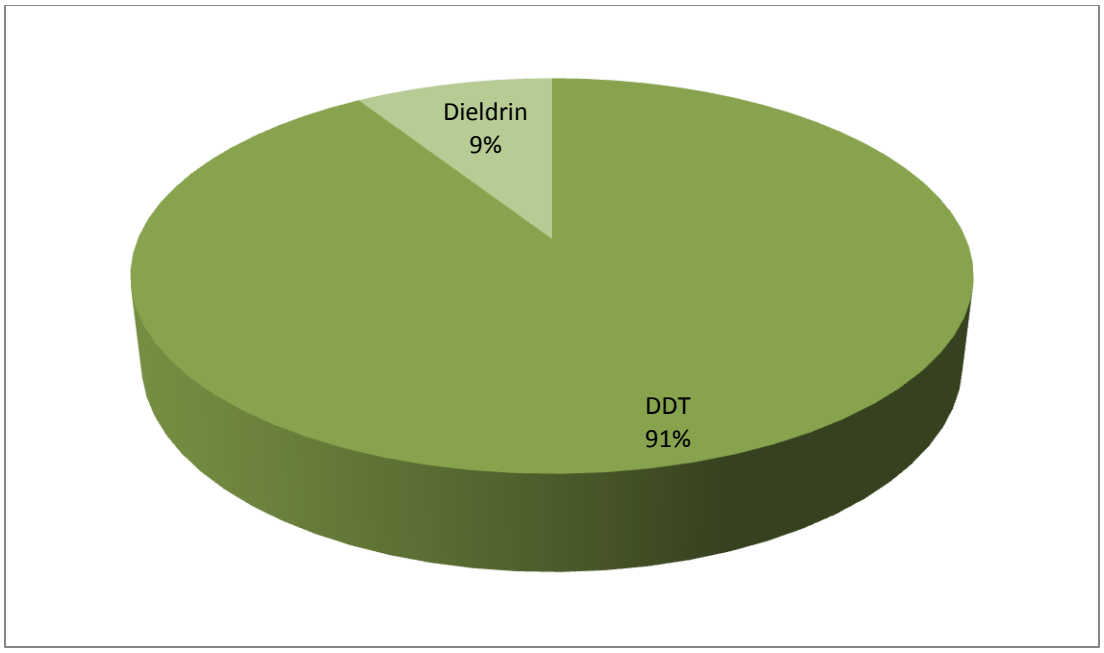


Figure 9: Resistance percentage against organochlorine insecticides in *Cx. quinquefasciatus*.

This unfortunate change of status from susceptible to resistance in just 7 years is an important matter of concern and presents a huge obstacle in tackling with the control and management of vector-borne diseases. Similar reports on DDT resistance in *Cx. quinquefasciatus* were recorded from Thailand (Somboon *et al.,* 2003), Benin (Corbel *et al.,* 2007), Sri Lanka (Wondji *et al.,* 2008), Zambia (Norris and Norris, 2011), Zanzibar (Jones *et al.,* 2012) and Ghana (Kudom *et al.,* 2013). The adult *Cx. quinquefasciatus* from Benin with 86-99% mortality (Corbel *et al.,* 2007)) showed a probability of the incipient insecticide resistance / susceptible status to be directed towards either way – resistance or susceptible. Though the application of DDT has decreased to medical sectors only, the existence of DDT resistance even today in mosquito vectors might be linked to the use of

the insecticide or to the phenomenon of cross-resistance with other organochlorines or with synthetic pyrethroids.

2.1.4. Resistance to carbamates:

Carbamate insecticides are a derivative of carbamic acid and are used for insect and other pest control in the household and agricultural practices because of its rapid killing effect. When compared to the other three groups of insecticides, reports on resistance/susceptibility status of mosquito vectors to this group of insecticide is few (Figure 10).

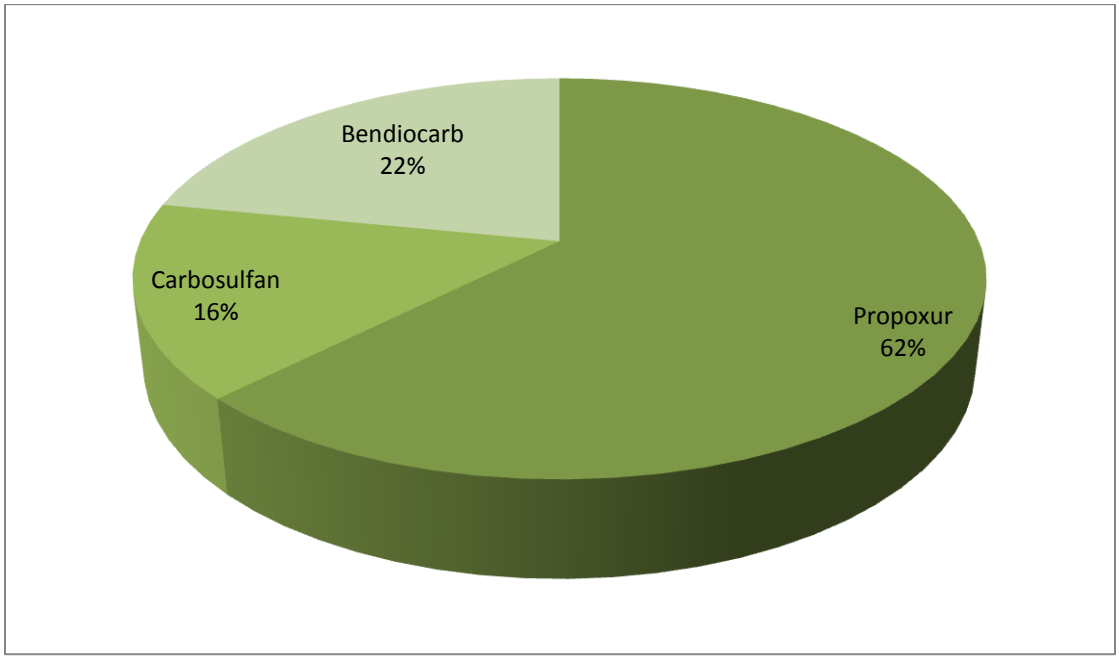


Figure 10: Resistance percentage against Carbamates in *Cx. quinquefasciatus*.

This might be because of lesser exploitation of carbamates in vector control programmes. However, due to its less toxicity on human skin and lower neurotoxic

properties as compared to the organochlorine insecticides, carbamates are also recommended for the purpose of mosquito control (WHO, 2016b). There are two broad applications of carbamate insecticides; first the use of this insecticide in the making of cockroach poison for household like various kinds of sprays and chalks. Secondly, carbamate is used along with synthetic pyrethroids in treating mosquito bed nets and also during indoor and outdoor residual spray programmes. Two prime carbamate insecticides used in mosquito control programmes worldwide are bendiocarb and propoxur.

In India, severe resistance to bendiocarb – a carbamate insecticide was reported from two states in adult *Cx. quinquefasciatus* with 5.7 mortality percentage in Raipur, Chhattisgarh and 16.2 mortality percentage in Kheda, Gujarat (Raghavendra *et al.*, 2011). The larval stages were also studied for their resistance/susceptible status to propoxur and was found to be resistant with LC_{50} values of 0.00013 ppm in Mysore (Kumar *et al.*, 2011) and 0.0001-0.1166 ppm in Bengaluru (Shetty *et al.*, 2013). The resistance to adult mosquitoes might be due to a cross-resistance phenomenon because of the use of other pest control products in the household and presence of carbamate insecticides in agricultural and aquatic environment because of its long residual effect. Apart from India, resistance to carbamate insecticides in *Cx. quinquefasciatus* is reported from several tropical and sub-tropical countries (Table 6).

Table 6: Insecticide resistance status of *Cx. quinquefasciatus* against Carbamates and Insect Growth Regulators (IGRs).

Sl. no.	Insecticides used	Life stages	Mortality (%)	Status	Country	References
1	Propoxur	Adults	____	____	Malaysia	Nazni *et al.*, 2005
2	Carbosulfan	Adults	30-75	R	Benin	Corbel *et al.*, 2007
3	Propoxur	Adults	77-93.3	R/IR	Thailand	Sathantriphop *et al.*, 2006
4	Propoxur	Adults	81.6-100	IR/S	Thailand	Thanispong *et al.*, 2008
5	Bendiocarb	Adults	5.7	R	India (Chhattisgarh)	Raghavendra *et al.*, 2011
6	Bendiocarb	Adults	16.2	R	India (Gujarat)	
7	Bendiocarb	Adults	52.4	R	Tanzania	Jones *et al.*, 2012
8	Propoxur	Adults	20	R	Malaysia	Chen *et al.*, 2013
	Bendiocarb	Adults	93.33	IR		
9	Propoxur	Adults	3.34-68.89	R	Malaysia	Low *et al.*, 2013b
10	Bendiocarb	Adults	12-100	R/IR/S	Ghana	Kudom *et al.*, 2013
11	Bendiocarb	Adults	52-76	R	Benin	Yadouletan *et al.*, 2015
12	Diflubenzuron	Larvae	0.0005*	R	India (Jodhpur)	Suman *et al.*, 2010
		Larvae	0.0006*	R	India (Bikaner)	
		Larvae	0.0003*	S	India (Jamnagar)	
		Larvae	0.0005*	R	India (Bathinda)	
	Iufenuron	Larvae	0.0006*	R	India (Jodhpur)	
		Larvae	0.0005*	R	India (Bikaner)	
		Larvae	0.0005*	R	India (Jamnagar)	

		Larvae	0.0006*	R	India (Bathinda)	
	Triflumuron	Larvae	0.0002*	R	India (Jodhpur)	
		Larvae	0.0002*	R	India (Bikaner)	
		Larvae	0.0002*	R	India (Jamnagar)	
		Larvae	0.0003*	R	India (Bathinda)	
13	Lufenuron	Larvae	0.000048*	S	Pakistan	Shah *et al.*, 2016
	Methoxyfenozide	Larvae	0.004*	S		
	Pyriproxyfen	Larvae	0.00008*	S		
	Cyromazine	Larvae	0.022*	S		
14	Propoxur	Larvae	0.00013*	____	India (Mysore)	Kumar *et al.*, 2011
15	Spinosad	Larvae	0.000002*	S	India (Mysore)	Kumar *et al.*, 2011
16	Propoxur	Larvae	0.0001-0.1166*	____	Bengaluru	Shetty *et al.*, 2013
17	Propoxur	Larvae	0.092-0.708*	____	Malaysia	Low *et al.*, 2013b
18	Propoxur	Larvae	0.116-0.389*	R	Bukina Faso	Skovmand and Sanago, 2018

*LC_{50} value in ppm; R: resistance; IR: intermediate resistance; S: susceptible

2.2. Mechanisms of insecticide resistance development:

Resistance development in insects against the insecticides is reported to occur through four different mechanisms: i) Behavioral resistance – where the insects avoid and deter the source of insecticide for direct contact in order to be safe from the toxic effects of the insecticides; ii) Cuticular resistance – the mosquitoes alter insecticide penetration by decreasing the intake of insecticides or penetration of chemicals through its surface, by increasing the thickness of the cuticle (Yahouedo *et al.*, 2017); iii) Metabolic detoxification through the increasing activity of major metabolic/ detoxifying enzymes and iv) Target-site insensitivity by alternation of the insecticide's binding site due to

various kinds of point mutations. The latter two mechanisms are considered as the most important mechanisms of insecticide resistance and have been widely studied.

2.2.1. Role of major detoxifying enzymes in insecticide resistance development:

Detoxifying enzymes or metabolic enzymes are naturally occurring enzymes in all living organisms, which protects an individual against the damage caused by xenobiotics and other metabolites that are endogenously produced. However, resistance to insecticides occur when there is an increase in the normal functioning of detoxifying enzymes. This increased activity of detoxifying enzymes lead to metabolic resistance in the pest population against insecticides and pesticides. Detoxification of insecticides in mosquito vectors and other insect pest due to increased enzyme activity has been linked to cytochrome P_{450} monooxygenases ($CYP_{450}s$), carboxylesterases (CCEs) and glutathione-S-transferases (GSTs) group of enzymes (Scott *et al.,* 2015; Soko *et al.,* 2015). This phenomenon of metabolic resistance in mosquito vectors develop due to over exploitation and uncontrolled use of insecticides against mosquito vectors and other insect and arthropod pests. Apart from synthetic insecticides, toxic compounds of plant source and other chemical pollutants also add up in the development of resistance in vectors and other insect pests.

Insecticide resistance through detoxifying enzymes in the mosquito vectors occur due to enhanced functioning of metabolic enzymes in preventing the insecticides from reaching their target through rapid transformation and excretion out of the body. This might occur in two ways either by enhanced activity of detoxifying enzymes due to a certain mutation that increases the detoxifying activity or by an increase in the copy number of a specific enzyme which results from an augment in transcription rate of the

enzymes. The detoxifying enzymes like GSTs, CCEs, and CYP_{450}s comprise of numerous genes belonging to supergene families that might have resulted from gene duplication in the course of evolution and there are even independent duplications specific to a particular species (Liu, 2015). On the molecular level of insecticide resistance, prominent query on mechanism lies on which gene or how many genes are actually responsible for directing the resistance phenotype; the type and number of mutations selected within the genes and whether these genes are appearing at multiple times or simply escalating from a single origin (Scott, 2019).

Any xenobiotic upon entering an insect body undergoes a series of enzymatic reactions that modify the polarity of the compound in order to make the compound water soluble for rapid and efficient excretion from the body (Hemingway *et al.,* 2004). This enzymatic action is divided into three phases of transformation and mainly includes the three major enzyme groups GSTs, CCEs and CYP_{450}s. Phase I reaction includes the multiple function oxidases enzymes: Mixed Function Oxidases or CYP_{450}s which functions by chemically modifying the xenobiotics by catalyzing its oxidation and creating a reactive site to which phase II enzymes bind. The modified products then enter phase II reaction where they undergo conjugation reaction brought about by the action of GSTs enzyme group. In phase III of transformation reaction, the modified water-soluble metabolites are finally excreted from the cells through ABC (ATP- binding cassette) transporters (Hemingway *et al.,* 2004). Hydrolysis of ester bonds in the xenobiotic compounds is carried upon by esterases and this group of enzyme actively participates in both phase I and II enzymatic reactions.

2.2.1.1. Carboxylesterases:

Enhanced activity of CCEs has been linked to organophosphate resistance in not only mosquito vectors but also other insect pests of medical and agricultural importance (Lopes *et al.*, 2019). In mosquitoes, this overproduction of non-specific CCEs is an evolutionary response, primarily to organophosphate insecticides and secondarily to carbamates though conferring resistance to pyrethroids as well in other insect pests (Gopalakrishnan and Veer, 2018). Organophosphate insecticides can be hydrolyzed by CCEs as these insecticides are esters of phosphoric acid. Usually, organophosphates are administered in its phosphorothionate form which in the phase I enzymatic reaction of xenobiotic transformation is converted to the active organophosphate form by Mixed Function Oxidases. The activated form is more toxic compared to the phosphorothionate form.

CCEs were earlier classified into two types. CCEs that were inhibited by paraoxon were termed as B esterases and those that were not inhibited by paraoxon were termed A esterase. In addition, B esterase have an active site serine residue. This nomenclature was modified in *Culex* mosquitoes based in the hydrolysis of α and β napthyl acetate and on the electrophoretic mobility of the enzyme (Hemingway *et al.*, 2004). Esterases that hydrolyzed α-napthyl acetate were termed Estα and those hydrolyzing β-napthyl acetate were termed Estβ. According to the mobility of CCEs on the native PAGE (Polyacrylamide Gel Electrophoresis), the enzymes were numerically labelled with the slowest one termed as Estα1 or Estβ1.

2.2.1.1.1. Quantitative resistance mechanism:

The organophosphate and carbamate insecticides in mosquito inhibit CCEs by rapid esterification of serine residue present on active site of esterases with the help of the insecticides' active oxon analogue. These insecticides have high affinity for CCEs but are poor substrates due to the slow rate of hydrolysis thus acting as esterase inhibitor. The binding of esterases to organophosphate and carbamate insecticides though rapid results in slow hydrolysis thereby making it a rate limiting step. In order to overcome this inhibitory action, CCEs act by rapid sequestration of the oxon analogues of insecticides. The term sequestration is rapid binding of CCEs to an insecticide and thereafter slow release of the metabolite. However, for this rapid sequestration to be carried upon, increased quantities of CCEs are needed because of the irreversible 1:1 stoichiometry of the enzymatic reaction and longer time taken for metabolism of the insecticide. Thus, mosquitoes resistant to organophosphate and carbamate insecticides possess huge quantities or over production of non-specific CCEs that prevent the insecticides from reaching the target site *i.e*., AChE (acetylcholinesterase) (Hemingway *et al.,* 2004).

The involvement of esterase in insecticide resistance in *Cx. quinquefasciatus* and other mosquito vectors can be detected classically by use of synergists like TPP (Triphenyl phosphate), DEF (S,S,S-tributylphosphorothioate) and IBP (S-benzylO,O-diisopropyl) along with the insecticide and thereafter recording the mortality rate of mosquito population. Presence of CCEs in resistant mosquito population can be detected quantitatively with the help of biochemical microplate assays or qualitatively through the use of native PAGE (Polyacrylamide Gel Electrophoresis) by using α- napthyl acetate or β- napthyl acetate as substrate (Hemingway and Karunaratne, 1998).

Overproduction of CCEs in insecticide resistance population of *Cx. quinquefasciatus* results from the amplification of genes that encode the esterase enzymes within the mosquito genome (Cui *et al.,* 2006). Moreover, this results in increase in the transcription rate of mRNA specific for the enzymes thereby producing high quantities of CCEs. Out of the many esterase genes and alleles that are associated with insecticide resistance in *Cx. quinquefasciatus,* the co-amplification of estα2 and estβ2 up to 80 times in highly resistant *Cx. quinquefasciatus* population is noteworthy (Paton *et al.,* 2000). This resistant genotype was found to be present in about 90% of *Cx. quinquefasciatus* populations that were resistant to insecticides. The close association of transposable elements or long interspersed repetitive elements (LINES) termed Juan-C with amplified Estβ1' have been reported from TEMR strain *Cx. quinquefasciatus* (Hemingway and Karunaratne, 1998). These transposable elements accelerate the frequency of mutation and amplification of CCEs genes and are influenced by environmental factors like insecticide selection pressure on the mosquito population.

Elevated activity of CCEs, Mixed Function Oxidases (MFO) and GSTs have been linked to the development of resistance to DDT, permethrin and carbosulfan in *Cx. quinquefasciatus* from Benin, Africa (Corbel *et al.,* 2007). Likewise, increased esterase activity detected in *Cx. quinquefasciatus* from Louisiana, United States was linked to naled (an organophosphate) resistance in the mosquito vector while suggesting other mechanisms of resistance towards synthetic pyrethroids and organophosphates (Gordon and Ottea, 2012). This observation was further supported by electrophoretic banding patterns, which revealed darkly stained bands in resistant mosquito vectors (Gordon and Ottea, 2012) and similar observation was reported from Pakistan (Tahir *et al.,* 2009). In a

study conducted in Ghana (Kudom *et al.,* 2015) elevated activity of both α and β esterases is correlated with DDT and synthetic pyrethroids resistance along with increased GSTs level in the mosquito vector.

Low *et al.,* 2013 reported a significant increase in esterase activity in *Cx. quinquefasciatus* with higher activity of α-est than β-est. This increase in enzymatic activity is linked to malathion resistance in the studied mosquito population and to organophosphate resistance (Low *et al.,* 2013a). On contrary to this finding, there was a significant correlation between the elevated activities of α and β esterase (Norris and Norris, 2011), which might be due to co-amplification of estα2' and estβ2' gene leading to organophosphate resistance in *Cx. quinquefasciatus* (Hemingway *et al.,* 2004). High CCEs activity is also a major mechanism of resistance development to DDT in mosquito vectors (Hemingway and Ranson, 2000; Sarkar *et al.,* 2009).

2.2.1.1.2. Qualitative mechanism:

Enzymes, which hydrolyze carboxylic ester bonds, are collectively termed as CCEs. Apart from the overproduction of non-specific CCEs for rapid sequestration of organophosphate and carbamate insecticides in few resistant populations of mosquito vector, rapid hydrolysis of insecticide occurs through elevated activity of CCEs. This mechanism of resistance involving CCEs is usually specific to malathion insecticide rather than a broad non-specific CCEs based resistance mechanism. This malathion-specific resistance mechanism of CCEs has been reported from malarial vector *Anopheles sp.* as well (Claudianos *et al.,* 2000). Karunaratne and Hemingway 2001 have reported the involvement of malathion-specific CCEs in development of malathion resistance in *Cx. quinquefasciatus* and other mosquito vectors like *Cx. gelidus*, *Cx. tritaeniorynchus*,

Anopheles culicifacies, *An. subpictus*, *Aedes aegypti*, *Ae. albopictus* with St. Mal strain of *An. stephensi* as a positive control for the study. Quantitative and qualitative mechanism of CCEs are not exclusive to one another as both have been found to occur in *Cx. tarsalis* (Hemingway *et al.*, 2004).

Though increased activity of CCEs is related to organophosphate and carbamate insecticide in *Cx. quinquefasciatus*, the esterases are reported to be ineffective against synthetic pyrethroids in the same vector. Moreover, a study reports negative correlation between increased CCEs activity and survival of filarial agent *Wuchereria bancrofti* in its vector *Cx. quinquefasciatus*. However, its effect on disease transmission, still remains vague.

2.2.1.2. Cytochrome P_{450} (CYP_{450}) Monooxygenases:

Cytochrome P_{450} (CYP_{450}) – dependent monooxygenases are haem containing hydrophobic metabolic enzymes that catalyze detoxification of a number of xenobiotics (Hemingway *et al.*, 2004) with an exception for the activation of organophosphate insecticides to its toxic oxon form. Apart from the involvement of CYP_{450}s monooxygenase in detoxification of plant toxins and insecticides, this enzyme group is also involved in the synthesis and degradation of insect hormones *i.e.*, ecdysteroids (Gilbert *et al.*, 2004) and juvenoids (Helvig *et al.*, 2004).

Elevated activity of CYP_{450} monooxygenases is reported to be linked to insecticide resistance in mosquito vectors (Hemingway *et al.*, 2004) along with other metabolic enzymes. The increased function of CYP_{450} monoxygenases is particularly related to pyrethroid resistance in mosquitoes. For example, elevated levels of CYP_{450}

monooxygenase has been reported from permethrin resistant *Anopheles gambiae* (Muller *et al.,* 2008), *Aedes aegypti* (Strode *et al.,* 2008) and resistant strain of german cockroach, *Blatella germanica* (Pridgeon *et al.,* 2003). In *Cx. quinquefasciatus*, there are various reports on the positive correlation of pyrethroid resistance and overproduction of CYP_{450} monoxygenases (Komagata *et al.,* 2010; Liu *et al.,* 2011; David *et al.,* 2013; Delannay *et al.,* 2018; Fagbohun *et al.,* 2019).

Several CYP_{450}s genes are found to be associated with insecticide resistance in mosquito vectors. Many studies have reported various genes involved in detoxification of insecticides and other xenobiotics. Earlier, *Cyp6* gene family of CYP_{450} monooxygenase was reported to be linked with elevated CYP_{450}s activity in response to insecticide resistance in insects. CYP6D1 protein was reported to be overproduced in pyrethroid resistant *Musca domestica* (Kasai and Scott, 2000). Cyp6z1 was overexpressed due to elevated transcription level in pyrethroid resistant *An. gambiae* population of East Africa (Nikou *et al.,* 2003). Likewise, *Cyp6f1* gene in permethrin resistant *Cx. quinquefasciatus* strain was reported to be involved in resistance development with elevated levels of *Cyp6f1* transcript (Kasai *et al.,* 2000). Another species of *Culex*, *Cx. pallens* was reported from China to be resistant to deltamethrin (Gong *et al.,* 2005). *Cx. pallens* showed the association of *Cyp4* gene family with deltamethrin resistance (Shen *et al.,* 2003). *Cyp4* gene family is reported to have the largest number of CYP_{450}s genes in *Drosophila melanogaster*, *An. gambiae* and *Ae. aegypti* (Strode *et al.,*2008). In deltamethrin resistant strain of *Cx. pipiens pallens* five *Cyp4* genes were reported to be overexpressed (Shen *et al.,* 2003). These genes are *Cyp4h21, Cyp4h22, Cyp4h23, Cyp4j4* and *Cyp4j6*. However, none of the genes are found to be upregulated and overexpressed in permethrin resistant

strain (JPal-per) of *Cx. quinquefasciatus* larvae (Komagata *et al.,* 2010). Similarly, *Cyp6f1* previously reported to be overexpressed in permethrin resistant strain (Kasai *et al.,* 2000) was not found to have significant overexpression in the same vector (Komagata *et al.,* 2010) and this might be due to change in expression pattern of permethrin resistant strain of *Cx. quinquefasciatus* over years of culture in the laboratory. Komagata *et al.,* 2010 reported elevated expression of three CYP_{450}s genes, *Cyp9m10, Cyp4h34,* and *Cyp6z10* in permethrin resistant *Cx. quinquefasciatus*. *Cyp4h34* and *Cyp9m10* were reported to show highest elevated levels in larval stages that decreased drastically in pupal and adult stages of permethrin resistant strain of *Cx. quinquefasciatus*. Hardstone *et al.,* (2007) reported higher resistance level in permethrin resistant *Cx. quinquefasciatus* larvae and a decrease in resistance level in adult *Cx. quinquefasciatus*. Thus, when correlating the findings of these two studies, it can be concluded that *Cyp9m10* and *Cyp4h34* monooxygenases genes are involved in pyrethroid resistance in permethrin resistant *Cx. quinquefasciatus* strain (JPal-per). However, overproduction of CYP_{450}s genes cannot be related with insecticide resistance always. This is because a single mutation occurring in transcription factor associated with expression levels of CYP_{450}s genes might also cause an overproduction of CYP_{450}s gene products without a stimulus of insecticide exposure.

2.2.1.3. Glutathione-S-transferases (GSTs):

Glutathione-S-transferases are dimeric proteins with a crucial role in metabolism, detoxification and excretion of various xenobiotic compounds. GSTs are soluble protein and are classified into three categories – microsomal, cytosolic and mitochondrial based on their location in the cell, majority of the GSTs being cytosolic in nature (Che-

Mendoza *et al.,* 2009). However, till date only microsomal and cytosolic GSTs are reported in insects. Cytosolic GSTs are the major GSTs group to be involved in insecticide detoxification (Claudianos *et al.,* 2006; Che-Mendoza *et al.,* 2009). Six different classes of insect GSTs have been reported in *An. gambiae* (Ranson *et al.,* 2002), among which Delta and Epsilon classes are the largest.

Cytosolic GSTs are primarily involved in the detoxification of both endogenous and exogenous compounds either directly or by secondary metabolism of wide range of compounds that are earlier oxidized by CYP_{450}s enzyme family (Enayati *et al.,* 2005). Cytosolic GSTs metabolize an array of hydrophobic and hydrophilic compounds by catalyzing conjugation of reduced glutathione (GSH) with the xenobiotic compounds like insecticides and reactive oxygen species (ROS) including superoxide anions, hydroxide radicals and hydrogen peroxide (Lumjuan *et al.,* 2007; Reddy *et al.,* 2011). As mentioned earlier, the general purpose of these enzymatic reactions remain an increase in the compound solubility for rapid excretion from the body (Ranson and Hemingway, 2005).

A GST subunit comprise of two domains i) N- terminus which has GSH binding site (G-site) and is a highly conserved domain; ii) C- terminus that provides hydrophobic substrate binding site (H-site) and is variable (Lumjuan *et al.,* 2007). Variation in the C-terminus of GSTs subunit results in diverse substrate specificity of the GST supergene family in insects. Moreover, few amino acid substitutions might result in dramatic change in the substrate specificity of GSTs family (Ortelli *et al.,* 2003)

An increase in the normal GSTs activity in insects has been linked to resistance to all four classes of insecticides in general (Hemingway *et al.,* 2004). The increase in GSTs

activity might be due to elevated amount of GSTs enzymes resulting either from an increase in the transcriptional rate or due to gene amplification. However, possibility of quantitative change in individual GSTs enzyme, which in turn results in elevated GSTs activity in insects have not been reported (Hemingway *et al.,* 2004).

Since its first identification in insect species, increased GSTs activity in insects has been linked to organophosphorous insecticide (Hayes and Wolf, 1988). Organophosphate insecticides are usually applied in non-toxic phosphorothionate form which are later in the insects's body converted to its active form by the action of CYP_{450}s. The GSTs are also involved in secondary metabolism of organophosphate insecticides as this enzyme family is reported to detoxify the active oxon analogue of organophosphate insecticides leading to organophosphate resistance in mosquito vectors with increased GSTs activity. Apart from organophosphate insecticides, GSTs play a role in the mechanism of DDT resistance also by catalyzing the dehydrochlorination of DDT thereby leading to resistance development in mosquito vectors to DDT (Hemingway *et al.,* 2004). Although GSTs are not directly involved in detoxification of synthetic pyrethroids, they catalyze detoxification of lipid peroxidation products that are produced by the induction of synthetic pyrethroids (Vontas *et al.,* 2001). As such, this enzyme group also play an important role in the onset of resistance to synthetic pyrethroids in mosquito vectors. GSTs might also sequester synthetic pyrethroids in the insect body and in turn protect insects from toxicity of synthetic pyrethroids (Kostaropoulos *et al.,* 2001).

In *Cx. quinquefasciatus*, elevated levels of GSTs have been recorded in populations resistant to DDT, carbosulfan and permethrin along with increased esterases activity (Corbel *et al.,* 2007). Similar involvement of GSTs in resistance development in

Cx. quinquefasciatus have been reported from Malaysia where elevated GSTs activity were observed for the first time on contrary to previous studies where post propoxur inhibition, there was no increase in the GSTs activity of *Cx. quinquefasciatus* populations (Low *et al.,* 2013b). Elevated levels of GSTs enzyme was reported as a secondary mechanism of resistance development against DDT, deltamethrin and permethrin in *Cx. quinquefasciatus* populations of Macha district in Zambia (Norris and Norris, 2011). Moreover, increased level of GSTs activity of the field collected population when compared to SLAB (laboratory reared susceptible) population was found to be correlated with DDT resistance in the *Cx. quinquefasciatus* population of Assam, India (Sarkar *et al.,* 2009). The result of enzyme activity level is also dependent on the age of mosquitoes used for the biochemical assay. With increasing age of the mosquito population, susceptibility towards insecticides is reported to increase (Rajatileka *et al.,* 2011). Decrease in the amount of detoxifying enzymes in older mosquitoes when compared to 2-3 days old ones is thought to be the reason behind such decrease in insecticide resistance (Rajatileka *et al.,* 2011). Elevated GSTs activity was also reported from Ghana in *Cx. quinquefasciatus* populations that were resistant to DDT and synthetic pyrethroids (Kudom, 2015).

2.2.2. Target-site insensitivity:

A mutation (point mutation) in the genes that encode proteins at the target-site of insecticides results in target-site insensitivity. Any structural modification in the target protein resulting from mutation in the genes encoding such proteins inhibit the normal interaction of target proteins with insecticides. The inability of applied insecticide to bind with a receptor at its specific target site results in the development of resistance due to

target-site insensitivity. Every insecticide on entering an insect's body bind to a particular receptor to exert its toxic effect on the insect. DDT and insecticides belonging to the synthetic pyrethroids group target the voltage-gated sodium channels (vgsc) in insects including mosquitoes. Binding of DDT and synthetic pyrethroids in the voltage-gated sodium channel results in prolonged opening of the channel. This prolonged depolarization of the nerve membrane causes the insect's nervous system to discharge repetitively resulting in paralysis and then death of the insect. Likewise, organophosphate and carbamate insecticides target the acetylcholinesterase (AChE) enzyme. AChE is a key enzyme in the nervous system which terminates nerve impulses by catalyzing the hydrolysis of acetylcholine neurotransmitters. Organophosphate and carbamate insecticides on binding with AChE enzyme inhibit the enzyme's activity by covalent phosphorylation by carbamylation of serine residues in the enzyme's active site. Inability of AChE to terminate nerve impulse in the insect's nervous system leads to continuous transmission of nerve impulse and ultimately death. Cyclodiene insecticides such as dieldrin and phenylpyrazzole insecticides such as fipronil target the GABA (ɤ-amino butyric acid) receptor. Binding of neurotransmitter GABA with its Type A GABA receptor causes rapid closure of the pentameric transmembrane chlorine channel. Insecticides like dieldrin and fipronil bind with GABA receptors thereby inhibiting the binding of neurotransmitter GABA with its receptor and gating of the chlorine channel. Any mutation in the GABA receptor leads to its structural modification and renders the protein unable to bind with insecticides. Therefore, structural modification of receptor proteins because of mutation in the genes that encode those proteins make the target

protein insensitive to the insecticides targeting those sites ultimately leading to development of resistance phenomenon in insects against the insecticides applied.

2.2.2.1. Voltage-gated sodium channel (vgsc):

The vgsc is a critical component of the insect nervous system and is required for the initiation and propagation of action potentials (electrical impulse). Action potentials across membrane are generated to conduct electrical information throughout the nervous system. When the membrane of excitable cell is at resting potential, the cell is in an inactive state and the sodium channel is closed. Upon activation, the cell membrane is depolarized leading to opening of sodium channel. Sodium channels open to allow the flow of sodium ions into the cell causing further depolarization of the membrane. This generation of transient sodium current results in action potential of the nerve impulse. However, after few milliseconds of channel activation, due to a conformational change in sodium channel, entry of sodium ions across the cell membrane is blocked. This process of fast inactivation is responsible for the falling phase of action potential (Dong, 2007) and when the membrane potential reaches its resting state, the sodium channel closes (Vais *et al.,* 2001).

Volatge-gated sodium channel (vgsc) comprises four homologous domains (I – IV) with six transmembrane helices or segments (S1 – S6) in each of the four domains. Most insects have a single copy of vgsc gene which encodes a protein – vgsc of ~2050 amino acids. Vgsc protein is a highly conserved protein and diversity in vgsc protein between species or between cells of an individual may result from mutually exclusive exons, exons with variable 3', 5' splice sites, optional exons, and RNA editing that produces sodium channels with different gating properties and neuronal excitability

(Scott, 2019). The vgsc protein diversity can also be measured by the identification of different number of haplotypes. Study upon vgsc diversity is important, as a vivid knowledge on vgsc variation will be fruitful in predicting the role of new mutations discovered in the development of insecticide resistance.

As the vgsc play an important role in the membrane excitability and transmission of nerve impulse, it is a target for many neurotoxins such as tetrodotoxin, batrachotoxin, and scorpion toxin produced either for defense or for predation (Wang and Wang, 2003). Insecticides belonging to synthetic pyrethroid class and DDT also target the sodium channel. Synthetic pyrethroids and DDT prolong the opening period of sodium channel by inhibiting channel deactivation and stabilization of channel open configuration resulting in prolonged membrane depolarization and ultimately death of the insect. DDT and synthetic pyrethroids modify the gating kinetics of sodium channel as these insecticides slow both the activation and inactivation of the channel (Hemingway *et al.*, 2004). Type II pyrethroids (like deltamethrin) prolong the opening of sodium channel during an action potential for a much longer time than Type I pyrethroids. However, the half activation potential *i.e.* the membrane potential where 50% of the sodium channels are open is greater on the application of Type I pyrethroids (Vais *et al.*, 2001). Because of the binding of pyrethroids and DDT with sodium channel receptor protein, depolarization of nerve membrane continues even when the cell is at resting potential thereby causing repetitive discharges of electrical impulse and eventually paralysis and death of the insect. Apart from synthetic pyrethroids and DDT, vgsc blockers (eg. oxadiazine indoxacarb and semicarbazone metaflumizone) are another group of insecticides that upon binding with the sodium channel protein causes slow activation of the channel and

impair the conduction of sodium ions thereby eventually causing death of the insect. Lack of cross resistance in the target site between these two different groups of insecticides suggest different role of synthetic pyrethroids/DDT and the vgsc blockers in mosquito vgsc (Shono *et al.*, 2004) though an exception has also been reported (Smith *et al.*, 2018).

Unrestrained use of insecticides since many decades, to control insects and other arthropod pests in both agricultural and health sectors have led to the development of resistance. A major mechanism of resistance due to intensive use of pyrethroid insecticides and DDT is the knockdown resistance (kdr). Kdr results from reduced sensitivity of sodium channels (primary target-site of synthetic pyrethroids and DDT) which arises due to point mutation(s) in the insect sodium channel. Decades of over exploitation of synthetic pyrethroids and DDT has led to kdr development in insects including the mosquito vectors and as such 61 mutations or combination of mutations in the vgsc protein has been reported (as reviewed by Scott, 2019) many of which confer resistance to synthetic pyrethroids and DDT. Rest of the mutations are yet to be tested for their ability to cause target-site insensitivity to synthetic pyrethroids and DDT (Scott, 2019).

Molecular analysis of the sodium channel revealed kdr mutation with a substitution of leucine by phenylalanine in the 1014 codon position in the sixth segment of domain II of the sodium channel gene in housefly *Musca domestica* and German cockroach *Blatella germanica* that were resistant to pyrethroids (Miyazaki *et al.*, 1996). After this first discovery of L1014F kdr mutation in pyrethroid resistant housefly and cockroach, the same mutation was also reported from various insect pests over the following years – diamondback moths *Plutella xylostella*, peach-potato aphids *Myzus*

persicae, western flower thrips *Frankliniella occidentalis* and Colorado potato beetles *Leptinotarsa decemlineata* (as reviewed by Dong, 2007). The kdr mutation was also reported from mosquitoes *Anopheles gambiae* (Martinez-Torres *et al.,* 1998), *Culex pipiens* (Martinez-Torres *et al.,* 1999) and *Cx. quinquefasciatus* (Xu *et al.,* 2005). Substitution of leucine by serine, histidine, cysteine and tryptophan (L1014F/H/C/W) has also been reported in the mosquito vectors (Scott, 2019). The substitution by serine in the 1014 codon position of sodium channel was earlier reported in *Cx. pipiens* (Martinez-Torres *et al.,* 1999) and in *An. gambiae* (Ranson *et al.,* 2000).

Eleven synonymous mutations have been identified in pyrethroid resistant mosquito vectors so far (Scott, 2019). These include, mutation from leucine to phenylalanine / serine / histidine / cysteine / tryptophan (L1014F/S/H/C/W), from isoleucine to methionine / valine (I1014M/V), from valine to glycine (V1016G), from phenylalanine to cysteine (F1534C), from serine to proline (S989P), from valine to leucine (V1010L), from asparagine to tyrosine (N1575Y) and from asparagine to tyrosine (N1794Y).

The common L1014F kdr mutation studied and reported in *Culex* and *Anopheles* mosquitoes was not found to be present in *Aedes aegypti*. Synthetic pyrethroids and DDT resistant strains of *Ae. aegypti* did not report any mutations at 1014 codon, the reason behind was regarded to be codon bias (Scott, 2019). This may be due to the need of simultaneous double nucleotide substitution at the 1014 codon in *Ae. aegypti* needed in order to have the required amino acids for kdr mutation and associated insecticide resistance. Instead of L1014 mutation, other kdr mutations such as V1016G, S989P,

F1534C, and V410L are in *Ae. aegypti* that confers resistance to synthetic pyrethroids and DDT.

The most common and widely studied L1014F mutation in *Cx. quinquefasciatus* associated with insecticide resistance to synthetic pyrethroids and DDT have been reported from different regions of the world and its presence has also been linked to resistance development in the vector population. Other substitutions like histidine and serine in place of leucine (L1014H/S) was reported from other mosquito vectors like *An. gambiae* and *Cx. pipiens* complex. However, these kdr mutations have not been reported in *Cx. quinquefasciatus* until date.

In highly resistant mosquito strains, combination of more than one mutations has been reported to co-confer insecticide resistance like S989P + V1016G in *Ae. aegypti*, V1010L + L1014S in *An. culicifacies*, L1014F + N1575Y in *An. gambiae* and V1016G + D1794Y in *Ae. aegypti* (Liu, 2015). Moreover, triple mutations in the sodium channel gene – S989P + V1016G + F1534C in *Ae. aegypti* is reported to cause highest levels of resistance to synthetic pyrethroids when compared to S989P + V1016G mutations (Scott, 2019) where a cut and paste pattern of mutations is suggested that causes an increase in the resistance level. In super kdr house flies *i.e.*, house fly strain exhibiting higher resistance to synthetic pyrethroids and DDT as compared to kdr houseflies, an additional mutation from methionine to threonine (M918T) occurs in segment IV-V of domain II of the vgsc along with the common L1014F mutation in segment six of domain II in the vgsc (Dong, 2007).

Collection of *Cx. quinquefasciatus* from fields having low insecticide resistance, susceptible strain and permethrin-selected strain having high insecticide resistance and studies conducted, showed the presence of nine mutations in the entire sodium channel. Three non-synonymous mutations, from alanine to serine (A109S), leucine to phenylalanine (L982F), tryptophan to arginine (W1573R) and six synonymous mutations are identified. Positive correlation between different levels of permethrin resistance observed in different mosquito strains and distribution of polymorphism frequency of both mutations suggest a great possibility of synonymous and non-synonymous mutations to be directly linked to not only the evolution of insecticide resistance but also to the inheritance of resistance pattern among insecticide selected generation of *Cx. quinquefasciatus*. The coexistence of nine mutations (synonymous and non-synonymous) in different strains of *Cx. quinquefasciatus* revealed 13 possible mutation combinations (Li *et al.,* 2012; Liu, 2015). Moreover, non-synonymous mutations apart from resistance development may be associated with different biological factors thus significantly altering gene functions, indulging in the formation of protein secondary structures, protein folding and interaction of protein with its substrate (Gupta *et al.,* 2000; Kimchi-Sarfaty *et al.,* 2007; Liu, 2015). However, synonymous mutations are believed to have no role in insecticide resistance as these mutations do not change the codon sequence and as such cannot alter the protein function (Kimchi-Sarfaty *et al.,* 2007). Apart from the large number of mutations in the vgsc and their association with resistance development against synthetic pyrethroids and DDT, the onset of resistance through inheritance because of mutation in vgsc is reported to be incompletely recessive (Scott, 2019).

2.2.2.2. Acetylcholinesterase (AChE) receptor:

Insensitive AChE – the target site of organophosphate and carbamate insecticides confer insecticide resistance in various mosquito species. Organophosphate and carbamate insecticides target AChE by inhibiting the enzyme's activity of terminating nerve impulses through hydrolysis of acetylcholine by covalent phosphorylation of the serine residue in active site of the enzyme. Insensitive AChE is reported to be the most common mechanism of resistance against organophosphate and carbamate insecticides in several mosquito species including *Cx. quinquefasciatus* (Hemingway *et al.,* 2004). Insecticide resistance in mosquito species due to insensitive AChE usually confers high carbamate resistance and comparably lower organophosphate resistance (Russell *et al.*, 2004).

Although two AChE genes *ace-1* and *ace-2* is reported in different mosquito species, which encode AChE1 and AChE2 proteins, resistance to organophosphate and carbamate insecticides in mosquitoes is linked to only AChE1 (Liu, 2015). In mosquitoes, two different types of mutations in AChE1 are reported until date to confer resistance to organophosphate and carbamates through insensitivity to the applied insecticides. Mutation in the 119 codon in AChE1 from glycine to serine (G119S) is reported to be associated with high levels of resistance to organophosphate and carbamate insecticide in *Cx. quinquefasciatus* and other mosquito species like *Cx. pipiens*, *Cx. vishnui* and *An. gambiae* as well (Liu, 2015). Glycine to Serine substitution in AChE1 protein causes steric hindrance that in turn reduces the affinity of AChE1 to its substrates and inhibitors thereby making the protein insensitive (Alout and Weill, 2008). The second mutation in AChE1 to confer resistance to organophosphate insecticides in *Cx.*

tritaeniorhynchus is substitution of Phenylalanine by Tryptophan (F by W) in 455 codon of AChE1 (F455W). Similar mutation (F455W) is reported to confer organophosphate and carbamate resistance in several insects thereby indicating the involvement and importance of this mutation in the development of insecticide resistance.

2.2.2.3. GABA receptors:

Cyclodiene insecticides such as dieldrin and phenyl pyrazole insecticide such as fipronil target the GABA receptor which is the binding site of neurotransmitter GABA that function as an agonist to open the pentameric transmembrane chlorine channel. A point mutation at 296-codon position in Rdl (resistance to dieldrin) gene in GABA receptor because of substitution of alanine by serine / glycine is reported to confer insecticide resistance in many insects including mosquito vectors (Liu, 2015). The A296S (alanine – serine) mutation is studied in *Anopheles* and *Aedes* mosquitoes and reported to cause dieldrin resistance (Du *et al.,* 2005) however, A296G (alanine – glycine) is reported from *An. gambiae* only (Du *et al.,* 2005). A296 mutation occurs in second transmembrane region of RDL subunit that forms the principal component of ion-channel and hence is associated with not only insecticide insensitivity but also to decreased desensitization rate (Hemingway *et al.,* 2004). In *Cx. quinquefasciatus* and *An. stephensi* resistance to fipronil is reported (Davari *et al.,* 2007; Liu *et al.,* 2005) despite the absence of any specific mutation in GABA receptor associated with fipronil resistance (Liu, 2015). This finding suggests the involvement of other mechanisms of resistance to fipronil in mosquito vectors. Moreover, similar mode of action and target site of both dieldrin and fipronil suggest the application of the latter insecticide with outmost

precaution keeping in mind the previously developed resistance against dieldrin in the mosquito vectors.

2.3. Biolarvicides:

Biolarvicides such as *Bacillus thuringiensis* var. *israelensis* (Bti) and *Lysinibacillus sphaericus* (Lbs) produce bacterial endotoxin that is activated by the mosquito larval intestinal proteases. The activated endotoxins bind to specific receptors of the epithelial lining of larval intestine gradually resulting in death of the larvae (Lopes *et al.*, 2019). Apart from its toxic effects on mosquito larvae, biolarvicides are applied for vector control because of their non-hazardous property towards the environment; high target specificity and low cost of management. Bti and Lbs are used for the control of mosquito vectors like *Cx. quinquefasciatus, Aedes sp.* and *Anopheles sp.* since the late 1980s. Lbs is generally used to control *Culex* and *Anopheles* mosquitoes and has a property of self-recycling in water with high organic content *i.e.*, an ideal breeding habitat of *Cx. quinquefasciatus*. Biolarvicides can be used to control mosquito species like *Culex* and *Anopheles* that have already developed resistance to chemical insecticides.

Compared to Bti, Lbs appears more efficient and promising in controlling *Cx. quinquefasciatus* as this bacteria is stable and has greater activity in polluted organic rich water where *Cx. quinquefasciatus* habitat. Lbs was applied in a large scale in Germany (Becker, 2000) and France to control *Cx. pipiens* that showed resistance to temephos, which is an organophosphate insecticide. It was applied in the tropical countries against *Culex* and *Anopheles* species in order to control the transmission of vector-borne diseases – malaria and filariasis as well. Recently Lbs was efficiently used to control *Cx.*

quinquefasciatus and *Ae. aegypti* populations in Columbia thereby paving an alternative pathway of vector control (Ahmed *et al.*,2007). However, the mosquito populations started developing resistance against biolarvicides as well. Resistance against Lbs has been reported in *Cx. pipiens* and *Cx. quinquefasciatus* since 1990s, first in laboratory selected *Culex* population and later in field treated populations from France, India, Brazil, China and Thailand (Charles and Nielsen-Leroux, 2005). However, there are no reports on resistance to Lbs in *Anopheles* populations and on resistance to Bti in mosquito vectors. In *Cx. quinquefasciatus cqm1* gene encodes a protein for intestinal epithelium and any mutation in the gene results in resistance against Lbs (Lopes *et al.,* 2019).

2.4. Cost of resistance development (Fitness cost):

Resistance developed against insecticides may cause some alterations in the normal physiology and life history traits of the insects. The primary aim of studying fitness cost of insecticide resistance in mosquito vectors is to examine the time-based resistance level in field populations of vectors that are exposed to insecticides (Belinato and Martins 2016). Information on the fitness cost of resistance and knowledge on which physiological parameters are affected by a particular resistance mechanism is important in designing better strategies of mosquito control. Several parameters like larvae development time, fecundity, chitin synthesis, life span, width of wings, blood feeding, adult longevity, mating competition, ability to avoid predators and the amount of ingested blood is usually studied for analysis of the fitness cost related to a particular resistance. *Cx. quinquefasciatus* populations that were resistant to synthetic pyrethroids showed longer larval development time when studied in the laboratory (Li *et al.*, 2002). Likewise, *Cx. pipiens* populations having resistant ace-1^R (insensitive AChE) and over production

of esterase (Ester^{1}, Ester^{4}) also showed a longer development time-period for larval growth (Belinato *et al.,* 2016). *Cx. pipiens* populations with resistant ace-1^{R} allele that codes for G119S mutation in the AChE, showed severe fitness cost as the frequency of resistant allele decreased on removal of insecticide selection pressure (Berticat *et al.,* 2004). There are also reports on organophosphate resistant *Aedes aegypti* populations showing low reproductive capacity as the resistant males fecundate few numbers of females only (Belinato *et al.,* 2012). Carbamate and synthetic pyrethroid resistant populations of *Cx. quinquefasciatus* mosquitoes showed cost of resistance development because fewer adult females emerged in insecticide-free environment as compared to the susceptible population (Berticat *et al.,* 2008). Moreover, the study also reported the compensation of fitness cost due to the presence of ace-1^{R} by Kdr^{R} allele (altered sodium channel gene conferring knockdown resistance) when present together in an insecticide-free environment (Berticat *et al.,* 2008). This is a major issue of concern and studies directed towards fitness cost associated with resistance in mosquito vectors has to be carried out in a more vigorous manner so that a definite strategy is build up in the control of mosquito vectors.